Title
The evolution of multiple mating: Costs and benefits of polyandry to females and of polygyny to males.

Journal
Fly, 6(1)

ISSN
1933-6934

Author
Gowaty, Patricia Adair

Publication Date
2012

DOI
10.4161/fly.18330

Peer reviewed

Fly 6:1, 3–11; January/February/March 2012; © 2012 Landes Bioscience

The evolution of multiple mating

Costs and benefits of polyandry to females and of polygyny to males

Patricia Adair Gowaty

Department of Ecology and Evolutionary Biology; Institute of Environment and Sustainability; UCLA; Los Angeles, CA USA; Smithsonian Tropical Research Institute; Washington, DC USA; Polistes Foundation, Inc.; Belmont, MA USA

Polyandry is a paradox: why do females mate multiple times when a single ejaculate often provides enough sperm for lifetime egg production? Gowaty et al. addressed explanations for polyandry in *Drosophila pseudoobscura* from the perspective of hypotheses based on sex differences in costs of reproduction (CoR). Contrary to CoR, Gowaty et al. showed that (1) a single ejaculate was inadequate for lifetime egg production; (2) polyandry provided fitness benefits to females beyond provision of adequate sperm and (3) fitness benefits of polyandry were not offset by costs. Here, I discuss predictions of the ad hoc hypotheses of CoR and three alternative hypotheses to CoR to facilitate a discussion and further development of a strong inference approach to experiments on the adaptive significance of polyandry for females. Each of the hypotheses makes testable predictions; simultaneous tests of the predictions will provide a strong inference approach to understanding the adaptive significance of multiple mating. I describe a sex-symmetric experiment meant to evaluate variation in fitness among lifelong virgins (V); monogamous females and males with one copulation (M_{OC}); monogamous females and males with multiple copulations (M_{MC}); P_{AND}, polyandrous females; and P_{GYN}, polygynous males. Last, I recommend the study of many different species, while taking care in choice of study species and attention to the assumptions of specific hypotheses. I particularly urge the study of many more Drosophila species both in laboratory and the wild to understand the "nature of flies in nature," where opportunities and constraints mold evolutionary responses.

Keywords: multiple insemination, cost of reproduction, compensation hypothesis, mating theory, switch point theorem, sexual conflict

Submitted: 09/01/11

Revised: 10/05/11

Accepted: 10/05/11

http://dx.doi.org/10.4161/fly.18330

Correspondence to: Patricia Adair Gowaty; Email: gowaty@eeb.ucla.edu

Extra View to: Gowaty PA, Kim YK, Rawlings J, Anderson WW. Polyandry increases offspring viability and mother productivity but does not decrease mother survival in *Drosophila pseudoobscura*. Proc Natl Acad Sci USA 2010; 107:13771–6; PMID:20643932; http://dx.doi.org/10.1073/pnas.1006174107

Multiple Mating in Drosophila

"Multiple mating" is mating with more than one potential mate, which potentially produces offspring of mixed parentage. Multiple mating is called "polyandry" when females do it and "polygyny" when males do it. In the vast majority of tested species, females mated in nature produce offspring sired by more than one male. Polyandry is common in wild Drosophila,[1-17] but also in crickets,[18] burying beetles[19] and other insects,[20] as well as birds[21] and mammals.[22,23] Compared with birds, in which studies of hundreds of species in the wild demonstrate the ecological correlates of genetic polyandry, generalities about Drosophila polyandry come mostly from laboratory studies, and most generalities about Drosophila polyandry come from only two species, *D. melanogaster* and *D. pseudoobscura*, though this is changing as more behavioral and evolutionary ecologists[24] ask questions about non-model Drosophila and Drosophila species in nature. Despite enormous success in understanding the ecology of polyandry in birds and its costs and benefits to wild-living females, much less is known about proximate causation in birds than in Drosophila. Almost all we now know of polyandry's proximate causes has come from Drosophila. A better understanding of the ultimate causes of polyandry in a variety of species of Drosophila, along with more studies of Drosophila in the wild, will close the

intertwining linkages of proximate and ultimate causation in the paradoxes of polyandry—at least for Drosophila—and provide additional direction for studies of polyandry in other taxa.

In laboratory studies of multiple Drosophila species, females often readily mate with more than one male.[15,17,25] Investigators often present experimental males to females sequentially rather than simultaneously, and this methodology has led to categorization of species as those with females re-mating at relatively fast vs. slow rates.[17] There are very few fly species in which most females mate just once.[17,26,27] In contrast, no studies of Drosophila from nature have demonstrated that specific males have sired offspring with more than one female, which might be explained by the methodological difficulty of assigning rather than rejecting paternity (assigning paternity is a problem not unique to Drosophila, but also in studies of other wild animals). In the laboratory, male Drosophila multiple mating is much easier to observe, especially under experimental mating schemes with sequential presentations of females to subject males; and, in the laboratory, male multiple mating is common.[13] Observations in some species suggest that female re-mating rate may be higher than male re-mating rate,[28] which may be associated with sex differences in development time, in turn affecting the operational sex ratio at eclosion sites where females often commence mating.[17] Even Bateman's[29] classic laboratory experiment with *D. melanogaster* suggested on reflection[30,31] and later re-analysis[32] that most females re-mated more than once, sometimes as often as most males in the experimental trials. Thus, the widespread general expectation derived mostly from laboratory studies of *D. melanogaster* that male Drosophila always re-mate more often than females re-mate remains speculation.

In this paper, I describe conceptual and theoretical ideas (**Table 1**) that together allow a strong inference[33] approach to current investigations of the costs and benefits to polyandry in species from any taxa, concentrating on the implications in studies of Drosophila. The ideas that I consider here have implications for future investigations of the fitness consequences for both females and males of multiple mating, i.e., of genetic polyandry and genetic polygyny. I also suggest an experiment for the future (**Table 2**), from the perspective of a balanced approach[34] to the constraints and opportunities for males[31,35] and females of multiple mating.

Polyandry Hypotheses

Hypotheses 1–5: Cost of reproduction. Anisogamy[36] and parental investment[37] theories are evolutionary explanations for fixed sex differences in pre-copulatory and copulatory mating behavior and physi-

Table 1. Comparison of fitness predictions for scenarios from cost of mating, compensation, demographic stochastic mating theory and the switch point theorem

		Component of Fitness				
	Polyandry hypotheses	Number of fertile eggs	Egg to adult survival†	Number adult offspring	Mother survival*	Adult offspring survival†
		M_{OC} M_{MC} P_M	M_{OC} M_{MC} P_M	M_{OC} M_{MC} P_M	M_{OC} M_{MC} P_M	M_{OC} M_{MC} P_M
1	[1]CoR: Guards against "sperm limitation"	$M_{OC} < M_{MC} = P_M$	No prediction	No prediction	No prediction	No prediction
2	[1,2]CoR: Decreases gamete incompatibilities	$M_{OC} = M_{MC} < P_M$	No prediction	No prediction	No prediction	No prediction
3	[1]CoR: Enhances offspring health	$M_{OC} = M_{MC} < P_M$	$M_{OC} = M_{MC} < P_M$	$M_{OC} = M_{MC} < P_M$	No prediction	$M_{OC} = M_{MC} < P_M$
4	[1]CoR: Enhances direct benefits to females	No prediction	No prediction	No prediction	$M_{OC} \leq M_{MC} \leq P_M$	No prediction
5	[1,2]CoR: Male coercion of remating by females	$M_{OC} = M_{MC} = P_M$	$M_{OC} = M_{MC} = P_M$	$M_{OC} = M_{MC} = P_M$	$M_O \geq M_{MC} \geq P_M$	$M_{OC} = M_{MC} = P_M$
6	[3]Compensation	$M_{OC} = M_{MC} < P_M$	$M_{OC} = M_{MC} < P_M$	$M_{OC} = M_{MC} < P_M$	$M_O \geq M_{MC} \geq P_M$	$M_{OC} = M_{MC} < P_M$
7	[4]Demographic stochastic mating theory	$M_{OC} \leq M_{MC} < P_M$	No prediction	$\leq \leq$	No prediction	No prediction
8	[5]Switch Point Theorem	$M_{OC} = M_{MC} < P_M$	$M_{OC} = M_{MC} < P_M$	$M_{OC} = M_{MC} < P_M$	$M_{OC} = M_{MC} = P_M$	$M_{OC} = M_{MC} < P_M$

Hypotheses for polyandry predict differences in components of fitness and the direction of effect comparing experimental treatments. The experimental treatments are (1) females with access to one randomly assigned male during only one day of their lives, i.e., monogamous females with one copulation MOC; (2) females with continuous access to a single randomly assigned male; i.e., monogamous females with multiple copulations MMC and (3) polyandrous females with access to a different randomly-assigned male (controlling for age and experience of males in MMC) on each day of the experiment; i.e., polyandrous females with multiple copulations, PMC. Read the entries thusly: MOC = MMC = PMC and so forth. *Mother survival is always expected to be lower for mothers with greater contact with conspecifics as required when females mate with more than one male, because of higher risk to pathogens and parasites, unless pathogen risk is offset by greater access to resources or other factors. †Offspring survival/per cohort is almost always expected to be higher under hetorozygosity, particularly at immune coding loci, which in many ecological circumstances will be higher when mothers have broods sired by more than one male. [1]Assumes the cost of reproduction CoR organizes sex differences, and for species in which eggs are larger than sperm, assumes females are choosy about mating, but males indiscriminate; assumptions and predictions are sex-asymmetric, differing for males and females. [2]CoR assumptions plus assume males are not just indiscriminate but coerce females into mating; and there are no additional benefits of multiple mating over single mating for females; asymmetric expectations for females and males. [3]Compensation assumes that offspring viability determines mate preferences, constraints on mating with one's most preferred exist and that under constraints individuals will attempt to compensate for offspring viability deficits. [4]Assumes demographic stochasticity affects opportunities for mating and all mating is "on encounter" (i.e., indiscriminate); symmetric expectations for females and males. [5]Assumes individual time available for mating and the likelihood of fitness conferred or lost from a particular mating determine whether an individual "accepts" a potential mate on encounter (is "indiscriminate") or "rejects" an encountered potential mate and waits for a partner with whom the individual will have higher fitness offspring (is "choosy").

Table 2. The design of a sex-symmetric experiment to evaluate the costs and benefits of multiple mating

Treatments (A through H)							
Alone adults		**Monogamy varied copulations**		**Multiple mating**			
Female	Male	Single copulation One-day exposure to partner	Many copulations Life-time exposure to the same partner	Female "Polyandry"		Male "Polygyny"	
				New virgin male daily	New sexually experienced male daily	New virgin female daily	*New sexually experienced female daily
A	B	C	D	E	F	G	H

With these treatments we will test the following predictions: (1) Assuming that the metabolic cost of producing eggs is greater than producing sperm, virgin females (A) die before virgin males (B). (2) Assuming that the cost to mating is greater in females than males, males live longer than females (D males > females). (3) Assuming that mating is costly, mated females die before virgin females (C females > A) and mated males die before virgin males (C males > B). (4) Assuming that female reproduction is limited by access to sperm, monogamous females who copulate once (C) lay fewer viable eggs than monogamous females who copulate repeatedly (D). (5) Assuming that polyandrous females have access to more variation in male haplotypes compared with monogamous females with constant access to males (D - while controlling variation in male age and experience), polyandrous females (F) have healthier offspring (% egg-to-adult-survival), more offspring who survive to eclosion and more adult offspring who survive longer. (6) Assuming that male quality varies with mating status, polyandrous females mated to a new virgin male every day (E) will lay more eggs than polyandrous females mated to sexually experienced males (F). (7) Assuming that multiple mating is associated with access to females with different alleles at immune coding loci, polygynous males (G) have healthier offspring than monogamous males – not controlling for female experience between treatments because males get a new virgin female everyday D males. (8) Assuming polygyny is costly for males, G and H males will die before D males. (9) Assuming that multiple mating is associated with access to more alleles at immune coding loci, polygynous males (H) have healthier offspring than monogamous males (D) while controlling between treatments for female age and mating experience, i.e., D < H. (10) Assuming pathogen risk is greater for polygynous than monogamous males, polygynous males (H) will die sooner than monogamous males (D) while controlling between treatments for female age and mating experience, i.e., D > H.

ology of males and females. Both anisogamy and parental investment assume that reproduction extracts predictable, deterministic costs that are greater in females than males, and thereby, in most species favoring choosy females and indiscriminate males. I call these arguments collectively "scenarios from the cost of reproduction" (CoR). CoR assumes the above selection arguments and predicts that selection acts differently on females and males such that male mating rate is much higher than female mating rate.

CoR arguments provide conceptual backing for Darwin's[38] observations and Bateman's[29] "principles" that (1) females are choosy and males indiscriminate about mating (2) which causes male variance in number of mates to be greater than female variance in number of mates so that (3) number of mates has a stronger effect on male reproductive success than female reproductive success.

Many investigators assume the veracity of the CoR arguments about choosy females and ardent males; yet very few experiments exist[39,40] that were designed to test under experimentally controlled conditions for the existence and magnitude of sex differences in pre-mating behavior. Nor, are there studies that evaluate the many alternative hypotheses, for what is called "courtship" by males; "courtship" could be attempts to persuade reluctant females to mate, or it could be, as it is in many bird and mammal species, a mechanistic way to coordinate male and female reproductive hormones; or it could be a type of communication serving collaborative functions between females and males both of whom have already indicated that the other is an acceptable mate. Instead of evaluating these alternatives, most investigators simply proclaim the generality of Darwin's observations, Bateman's conclusions[41] and CoR's predictions. Thus, they expect the optimal mating frequency for females to be once or always less than males, particularly in species in which females store sperm. Under CoR assumptions, it is unlikely that mating with more than one male increases female reproductive success. CoR scenarios predict that males provide to females in a single ejaculate enough sperm to fertilize their entire lifetime of egg production. And, under most characterizations of CoR assumptions, but not all,[35] whenever CoR is lower for males than females, males are predicted to be ardent and enthusiastic about mating, while females are predicted to be retiring, coy, relatively passive and choosy about mating.

Observations inconsistent with CoR. On the face of it, the very existence of polyandry rejects CoR. When there are no survival differences between monogamous and polyandrous females, one must seek other explanations for sex differences in mating behavior. Yet, only a very few investigators have considered that non-confirmatory empirical observations are actually a challenge to CoR ideas. Confronted with empirical inconsistency, many investigators instead cast ad hoc hypotheses of, e.g., "requisite" sexual conflict from the "coy female, ardent male" imperative to explain contrary data. In addition to evidence inconsistent with CoR, many criticisms of the CoR assumptions exist: it is unsurprising that there are many ad hoc adjustments to the basic CoR assumptions.

There are five CoR polyandry hypotheses (**Table 1**) with predictions about the benefits and costs of polyandry. The predictions vary depending on additional assumptions about what fitness benefits to females, either direct or indirect, organize female choices of mates. For example, if female mate choice only guards against sperm limitations (hypothesis 1), such as insufficient or defective sperm or incompatibilities between gametes (hypothesis 2), the hypothesis predicts that the number of eggs laid is less for females who copulate one time rather than multiple times, independent of whether the multiple copulations are with the same male.

Discerning between hypothesis 1 and hypothesis 2 when there is empirical consistency with their predictions requires additional mechanistic studies to evaluate the cause(s) of variation in egg number.

If one assumes that females choose mates for enhanced offspring health benefits, (hypothesis 3) the CoR predicts higher number of eggs laid, higher egg to adult survival, more adult offspring, and healthier adult offspring for polyandrous than monogamous females, whether monogamous females mate more than once or not. The basic costs of egg production under CoR predict that polyandrous females die faster than monogamous females.

If one assumes that females choose mates for direct benefits (hypothesis 4), the CoR predicts no differences in number of eggs laid, in egg to adult survival, number of adult offspring, and survival of adult offspring for monogamous and polyandrous females. Hypothesis 4 does predict, however, a survival benefit for those females who mate more often, whether they are polyandrous or monogamous, copulating multiple times with the same male.

Hypothesis 5: Sexual conflict and polyandry. The ad hoc sexual conflict hypotheses often characterize female multiple mating as an interaction between two or several competing males inside the bodies of females where sperm battle, as though the females are jars. The inside-female mechanisms by which males may decrease the reproductive success of rivals sometimes extract costs to females,[42] sometimes decreasing their survival probabilities and making tendencies for female re-mating to be inhibited.[43-48] In this view, females are caught in the cross-fire of male rivals, so that the cause of female re-mating is only to reduce costs extracted from pre-mating chasing, so-called "courtship" harassment, or post-mating collateral cost, not to gain some intrinsic fitness rewards from the mating. The costs to females of post-copulatory sperm wars are through accessory peptides that increase female egg-laying and negatively affect females' survival probabilities, which provides for some scenarios, the only selective force of female resistance,[49,50] which as some have noted could indeed have huge effects on the evolution of both female and male traits.

The "male coercion of females" sexual conflict hypothesis (hypothesis 5) predicts (**Table 1**) that egg-to-adult survival, number of adult offspring, and survival of adult offspring will be statistically similar whether females mate monogamously or polyandrously. Hypothesis 5 further predicts in most characterizations of the hypothesis that the more females mate the more likely they are to die.

Alternatives to CoR assumptions. It is also possible, but not yet experimentally investigated, that male mechanisms affecting females' reproductive decisions are "nice" rather than "nasty";[49] being things that may enhance, rather than decrease, female survival. This, according to the switch point theorem[34] (discussed below), would make females ever "choosier" and less likely to accept encountered potential mates in the future. And if there are independent benefits for females of multiple mating that drive their multiple mating in the first place, what might this mean to how we theorize the fitness dynamics of female polyandry? Positive effects on females may arise because competing rivals theoretically may also donate with their ejaculates nutritive or immune enhancing elements to females.[51,52] And these, of course, may enhance female fitness directly.[52] Negative effects of ejaculate donations on females are more commonly discussed; but the existence of negative effects does not mean that positive effects do not also occur, as they do.[27]

Alternatives might start from the first principle: sex is a necessarily collaborative process between two individuals sharing gametes.[49,53,54] Or, one might start with the idea that females have power too.[49,55] Could it be that females who multiply mate in the first place organize and manipulate male contests for their own advantage?[56-58] Why not imagine that females make the post-copulatory rules of the inside games through their physiological organization of the rules and their responses to males? Why not cast females not as umpires but players in their multiway interactions with males? Indeed, why not hypothesize that females are "the architects of sperm competition,"[59] a reasonable alternative hypothesis, for which supportive data exist.[60,61] Why not imagine that the dynamic interactions and their outcomes between females and males lead sometimes to reversals of power between the sexes, and over time, to sex-symmetric power distributions?[49] Why not imagine a dance of symmetric jousting over the, only sometimes, competing, but often necessarily cooperative[62,63] interests of females and males?

If no fitness rewards for females of multiple paternity exist, the problem of female multiple mating might indeed be entirely a problem of male-male competitive interactions: which male is the best competitor or which male extracts the least harm. However, if offspring viability (i.e., offspring health) matters to prospective parents, for example, alternative potential mates will often offer females a better or worse shot at healthy offspring.[34,62,64] These alternatives have occasionally been tested,[65,66] but seldom in the context of female lifespan variation,[67,68] making it very hard to discern some selectively effective costs. Assuming that there exist fitness rewards for females of controlling their own reproductive decisions, male competitive interactions pre- or post-mating put females in exquisite binds in which the benefits and costs of polyandry are in dynamic tension, so that female resistance to male control attempts will evolve[49] in evolutionary time or be induced in ecological time.[69]

Hypothesis 6: Compensation theory says multiple mating enhances offspring health with mutual benefits to both parents. If the pathogens and parasites of the offspring generation are different from those of the parental generation, as expected when pathogens and parasites evolve more rapidly than their hosts, the "Red Queen's Challenge" to parents[62] is how to produce the best immune systems in their offspring. Consideration of basic rules of inheritance of parental alleles means that in general parents will produce offspring with excellent immune function against the parent generation pathogens.[62] What can parents do to increase the likelihood that offspring will have the rare alleles and rare phenotypes that will enhance their survival in the face of novel disease risks that arise in the offspring generation? One route would be mutual

mate choice, in which both females and males choose mates complementary (i.e., dissimilar) to themselves at immune coding loci.[34,62,63] This option probably works for large, viscous populations with few social or ecological constraints on mate encounter; however, ecological and social impediments to free mate encounter are ubiquitous, probably vary by species, and suggest that most parents are making the best of a bad job, providing selection favoring variation in parental effects to increase the likelihood that offspring survive long enough to enter the ranks of breeders.

The compensation hypothesis (CH)[62] (hypothesis 6) says that both female and male partners will be under selection waged via the success of other parents with healthier offspring to compensate for expected offspring viability deficits. In *D. pseudoobscura*, both females and males discriminate among potential mates,[39,40] and when male or female choosers are in enforced experimental pairs with a potential mate they prefer compared with one they do not, the pair produce more adult offspring (but not more eggs) than when the chooser is with a potential mate they did not prefer.[70] These observations are inconsistent with the CoR prediction of indiscriminate male *D. pseudoobscura,* which have the smallest sperm of any known Drosophila species. More interestingly, as the CH predicted, females with males they did not prefer laid more eggs than females with males they did prefer. As a result, even though their offspring had lower egg-to-adult survival, some of the offspring survived to breeding age, keeping the lineage of compensating females alive. Laying more eggs to increase the likelihood that some survive to breed is a known compensatory mechanism, and may levy costs for both sexes of parent when environments are challenging.[62,63] Similar compensatory mechanisms occur in several model vertebrate species as well as female *D. pseudoobscura*.[63] Likewise, CH predicts that polyandrous mating provides compensatory benefits to females mating under ecological or social contraints.[62]

Many mechanisms of compensation are possible; some compensatory mechanisms are available for both females and males; others are sex specific. Elementary selection logic suggests that both sexes might compensate in cooperative efforts to enhance the likelihood of survival of their offspring. In species like flies in which post-zygotic parental care is unlikely and unknown, there are nonetheless many options that individual parents might use facultatively or flexibly to enhance offspring survival. From the perspective of CH the remarkable observations of Markow and her collaborators of male elemental contributions to ovarioles,[52] ejaculate-associated nutritive contributions to females and their offspring[51] are particularly interesting. Could the long-tails of sperm in the giant sperm species sometimes be nutritive as well? Do males contribute more when they mate with females with whom they will produce less viable offspring? Are these mechanisms ones that could be flexibly upregulated in some matings but not others?[54] These questions about the options some males have to collaboratively attempt to enhance the reproductive performances of their mates or even the health of their mates are intriguing, and as far as I have been able to tell, uninvestigated in terms of their effects on fitness variation of parents. Perhaps this is not strange given the current hegemony of sexual conflict and sexual antagonism dominating discussions of the relationships between the sexes. Novel insights likely await newer investigations of the nature of these potentially collaborative interactions between males and females in species like flies that lack post-zygotic parental care.

If multiple mating is a mechanism of compensation for offspring viability deficits, the CH predicts (**Table 1**) egg to adult survival and adult offspring survival is greater in monogamous than polyandrous mating, but that the number of adult offspring for monogamous and polyandrous females is the same, and furthermore that polyandrous females die sooner than monogamous females, not just because of enhanced exposure to conspecifics, but because of the costs of compensation.

Hypothesis 7: Demographic stochastic mating theory predicts polyandry and polygyny are common in most species. In contrast to the intuitive scenarios above, the axiomatic mating theorem of Hubbell and Johnson[71] proved theoretically that multiple mating is common in both sexes. Assuming large population sizes, demographic stochasticity (by chance some individuals die or otherwise leave or enter populations), and no selection pressures against multiple mating, the analytical solution of demographic stochastic mating theory (DSMT) says that individual encounter probability (*e*) with potential mates, individual survival probability (*s*), and the time after mating to receptivity to remating (*l*) determine lifetime mating rates. Under the simplifying assumptions of DSMT, differences between individuals and the variance among individuals in their lifetime mating rates arise from differences in the time they have available for mating,[64] modulated in all individuals of either sex by *s*, *e* and *l*. Thus, multiple mating in either sex can evolve in the absence of fitness benefits for either sex. From the perspective of DSMT, female multiple mating is no more problematic than male multiple mating: "it's all about time"[64] and the ecological and social constraints affecting individuals' time available for mating and reproduction.

The DSMT (Hypothesis 7) predicts that there are no differences in egg to adult survival or in adult offspring survival between monogamous and polyandrous females (**Table 1**), and that the number of adult offspring is greater than or equal between monogamous and polyandrous females. Without additional assumptions DSMT makes no predictions about lifespan variation of monogamous and polyandrous females.

Hypothesis 8: The switch point theorem predicts that multiple mating by either sex is adaptive. The DSMT background certainties of probabilistic variation in *e, s* and *l*, which produces real-time changes in the time an individual has available for mating[34,64,69,71] must also shape fitness enhancing mating decisions. For this to be so, females and males need to discern fitness costs and benefits of mating with this one or that one, given the unique demographic circumstances of each decision-making individual. If individuals discern fitness costs and benefits, the switch point theorem (SPT)[34] shows how adaptively flexible individuals evolve so as to maximize their fitness in contemporary time; trading off their time available

for mating with fitness, so that they sometimes accept and sometimes reject a mating opportunity. The SPT proved theoretically that males and females trade off time with fitness gains as they make "flexibly adaptive" decisions to accept or reject a mating.

If individuals make flexible reproductive decisions to accept or reject so as to optimize their fitness given specific demographic circumstances, as the SPT (Polyandry hypothesis 8) says, there will be differences in fitness outcomes among individuals, but these need not be, in nature, systematic benefits or costs to multiple mating relative to single mating by either sex (**Table 2**). More important, the SPT implies that there is no necessary sex differential cost of multiple mating over the cost of single mating for either sex (**Table 2**). That there is no further extracted cost in terms of survival of multiple mating for females is a unique prediction of the switch point theorem (**Table 1**).

Interpreting Results of Gowaty et al.

Gowaty et al. demonstrated that monogamously mated females with multiple copulations (M_{MC}) had more surviving offspring than monogamous females mated one time (M_{OC}), because single mated females ran out of sperm before they died or that sperm were otherwise inadequate. Compared with M_{MC} females, P_{MC} females with access to a new male every day, and who could have mated with each one and did mate with more than one male, had offspring with significantly higher egg-to-adult survival and significantly more offspring that survived to eclosion. Despite our a priori intuition that exposure to more conspecifics would decrease P_{MC} females' health status and thus, their lifespan compared with M_{OC} and M_{MC}, there were no significant differences in lifespan among the three types of experimental females.

All females entered the experimental treatments at the same time. All females and males were virgin at the start of the experiment. We kept trial sets consisting of "paired" females who were M_{OC}, M_{MC} and P_{MC} together throughout the experiment to control for bench effects. We controlled for the ages and prior experience of males interacting with M_{MC} and P_{MC} females by rotating the males in the P_{MC} treatments between other P_{MC} females. The study (Gowaty et al., 2010) was simple and tractable, but large and time consuming, with the oldest female dying at 138 d old, 131 d after the start of the experiment. We made the following conclusions: (1) polyandrous females produced offspring with higher probabilities of survival than that of offspring of monogamous females; and (2) polyandrous females did not die significantly faster than monogamous females. I considered the study important for two reasons. First, it solved the puzzle, "What do females want"—for *D. pseudoobscura*, at least. Because we eliminated the possibility of multiple-male pre-mating harassment of females, the conclusions were unambiguously associated with females' mating with multiple, different males compared with one male. Second, it showed—to our surprise and for the first time in any Drosophila species—that exposure to and mating with more than one male was not costly to females.

Gowaty et al. did not dwell on the ramifications of the failure of our observations to match the predictions of the CoR and sexual conflict scenarios, nor its consistency with the predictions of compensation, DSMT or the SPT. The data provided no support for egg-laying prediction of hypothesis 1 about sperm limitation or hypothesis 2 about gamete incompatibilities. Our data are inconsistent with hypothesis 4 about direct benefits to females of multiple mating, and our data reject hypothesis 5 of male coercion of female remating. The data were inconsistent with the hypothesis 6, the CH, but CH advocates might argue that the comparison of costs and benefits of polyandry were an inadequate test of the underlying assumptions of the CH, and thus logically not a test of the compensation hypothesis. The data over all were consistent with hypothesis 7 DSMT, however, DSMT could not account for the enhancement of offspring viability via polyandrous mating—at least without an add-on ad hoc assumption. Overall, however, the data show explicitly that polyandry benefited offspring health, consistent with hypothesis 3 about offspring viability and hypothesis 8, the SPT. This is not surprising as both of these ideas depend on an assumption of offspring viability as the main component of fitness organizing mating behavior; i.e., these two hypotheses are not alternatives and can be simultaneously so.

The discussion above stresses the significance of the assumptions of hypotheses. Clearly, in order to explore further the origins and benefits of multiple mating for females, our collective attention should be on the various assumptions of these hypotheses. Future strong inference crucial tests[33] of these hypotheses will depend upon the veracity of the assumptions of each hypothesis. Thus, surely it is the assumptions of the various hypotheses that future investigators should attend to.

Next Questions

Mechanism and strongly inferential tests of predictive theories of multiple mating in females and males. Gowaty et al. did not investigate the mechanistic bases for the study results, or whether pre-touching mate assessment or preferences predicted fitness; we asked no questions about mechanisms of sperm competition, physiological post-ejaculatory female resistance, variation in ejaculate secondary compounds and their possible negative or positive effects on females, zygotes and offspring were not part of our study. The study addressed only the "ultimate" questions of fitness variation of alternative mating scenarios, not the "proximate" mechanisms, except for the proximate "ecological" cause of availability to females of males who were potential mates (in two treatments females had access to only a single male and in the other multiple males). We necessarily left questions of proximate cause for others, not because we are uninterested. Simultaneous study of ultimate and proximate causes is an ideal, but constraints on funding and time seldom allow, perhaps never have allowed, simultaneous study of proximate and ultimate questions, particularly in demographic context.

Sexually symmetric tests of fitness payouts for multiple mating. Gowaty

et al. was necessarily silent about fitness costs and benefits for males, yet a symmetric study of female and male subjects would have been much more powerful in evaluating the intuitive CoR ideas, which "begin with sex differences to predict further sex differences." However, my colleagues and I have a track-record of completed previous sex-symmetric studies using *D. pseudoobscura* showing the following: (1) both females and males make pre-mating assessments of potential mates.[70] (2) For individual males and females, pre-mating assessment (preference) behavior predicts reproductive success, including offspring health and the number of offspring that eclose.[70] (3) Lifespan variation for males whom females preferred or did not prefer and for females whom males preferred or did not prefer were statistically indistinguishable, consistent with the conclusion that there were not intrinsic "quality differences" between potential mates who were preferred or not preferred.[72]

Gowaty et al. violated our tradition of sex-symmetric studies in our study of female, but not male, multiple mating. So, what would a simultaneous test (**Table 2**) of fitness costs and benefits for female and male subjects possibly tell us? Controlling for notorious "local bench," diurnal and seasonal effects, simultaneous tests allow a fair comparison between the sexes. It would level the playing field by asking the same questions of males as we ask of females, in the same way, so that the experimentally manipulated constraints acting on our subjects, whether female or male, would be the same. It would decrease the likelihood that subtle biasing factors associated with inevitable intuitions–about what males or females ought to do–from creeping in unnoticed. And, simultaneous testing of costs and benefits of male multiple mating would give our best chance of evaluating the effects of social and ecological constraints on individuals of either sex.

Next Steps: To Wild Nature

Connecting laboratory studies of fitness variation to real-world vagaries of the wild, where social opportunities and ecological constraints mold evolutionary response of Drosophila sex and evolution, is no easy task,[24] perhaps especially now that we know that developmental plasticity and epigenetic responses are so important to phenotypic evolution.[73] Arguing that laboratory experiments about fitness variation are beside the point misses the truth of what laboratory experiments are good for: namely they give us a precise understanding under specifically controlled conditions of how individual reproductive success and survival might vary, when many other confounding factors are removed. In the case of the polyandry experiment, we removed the confounding factors of pre-mating male-male competitive interactions, as well as overlooked, female-female competitive interactions, and potentially coercive inter-sexual interactions, such as multi-male harassment of females and mating pairs. In the idealized world, where these sorts of constraints were removed, we are able to make strong conclusions about the fitness effects of different kinds of social exposures. Are these effects wiped out, one might ask, in the more realistic settings of nature, where hurricanes, predator risk and variation in food resources and mates might vary? Probably. But then we are left with only correlational explanations limited to variables we are able to measure and have measured in the wild. What we can hypothesize about such interesting phenomena as giant sperm, ejaculate-associated nutritional donations to females and offspring, ejaculate accessory proteins, or explanations for why within species some females lay more or fewer eggs than others are enriched with careful laboratory evaluations of many hypotheses, including particularly first principle, axiomatic, quantitative theory that most often yield highly vulnerable crucial predictions. Starting in the laboratory is probably a very good place to begin, but not a good place to end. Our knowledge about flies in nature would be enhanced with efforts to connect the intriguing results of lab experiments on social behavior—fitness[74] and mechanism—to what happens in the wild.

Acknowledgments

I thank Stephen P. Hubbell and Allen J. Moore for comments on a previous version of the manuscript. I thank Wyatt W. Anderson and Yong-Kyu Kim for years of interesting collaborations on social behavior and fitness of Drosophila. This work was partially funded by an NSF grant (IOS 0911606).

References

1. Anderson WW. Frequent multiple insemination in a nutural population of *Drosophila pseudoobura*. Am Nat 1974; 108:709-11; http://dx.doi.org/10.1086/282949
2. Cobbs G. Multiple insemination and male sexual selection in natural populations of Drosophila pseudoobscura. Am Nat 1977; 111:641-56; http://dx.doi.org/10.1086/283197
3. Levine L, Asmussen M, Olvera O, Powell JR, Delarosa ME, Salceda VM, et al. Population genetics of Mexican Drosophila 5. A high rate of multiple insemination in a natural population of *Drosophila pseudoobscura*. Am Nat 1980; 116:493-503; http://dx.doi.org/10.1086/283644
4. Loukas M, Vergini Y, Krimbas CB. The genetics of drosophila subobscura populations. 18. Multiple insemination and sperm displacements in *Drosophila subobscura*.. Genetica 1981; 57:29-37; http://dx.doi.org/10.1007/BF00057540
5. Akin E, Levene H, Levine L, Rockwell R. A conservative procedure for the estimation of multiple insemination in Drosophila. American Naturalist 1984; 124:723-37.
6. Williams SM, Strobeck C. Measuring the multiple insemination frequency of Drosophila in nature: use of a Y-linked molecular marker. Evolution 1986; 40: 440-2; http://dx.doi.org/10.2307/2408827
7. Levine L, Akin E, Olvera O, Gaso MI, Rockwell RF, Gonzalez F, et al. Chromosomal and behavioral studies of Mexican Drosophila. 5. Frequencies of multiple insemination in 3 natural populations. Am Nat 1987; 129:458-62; http://dx.doi.org/10.1086/284650
8. Kambysellis MP, Craddock EM. Studies of oogenesis in natural populations of Drosophilidae. 4. Insemination patterns in hawaiian Drosophila species (Diptera, Drosophiliadae) correlated with ovarian development. J Insect Behav 1991; 4:83-100; http://dx.doi.org/10.1007/BF01092553
9. Aspi J, Lankinen P. Frequency of multiple insemination in a natural population of *Drosophila montana*. Hereditas 1992; 117:169-77; PMID:1459857; http://dx.doi.org/10.1111/j.1601-5223.1992.tb00171.x
10. Quezada-Díaz JE, Santos M, Ruiz A, Fontdevila A. The evolutionary history of *Drosophia buzzatti*. 25. Random mating in nature. Heredity 1992; 68:373-9; PMID:1563969; http://dx.doi.org/10.1038/hdy.1992.53
11. Snook RR, Markow TA. Efficiency of gamete usage in nature: sperm storage, fertilization and polyspermy. Proc Biol Sci 2002; 269:467-73; PMID:11886638; http://dx.doi.org/10.1098/rspb.2001.1854
12. Yenisetti SC, Hegde SN. Remating in a Drosophilid: Phorticella striata. Kor J Genet 2002; 24:113-8.
13. Markow TA, Sawka S. Dynamics of mating success in experimental groups of Drosophila melanogaster (Diptera, Drosophilidae). J Insect Behav 1992; 5:375-83; http://dx.doi.org/10.1007/BF01049845

14. Gromko MH, Markow TA. Courship and remating in field populations of Drosophila. Anim Behav 1993; 45:253-62; http://dx.doi.org/10.1006/anbe.1993.1031
15. Markow TA. Evolution of Drosophila mating systems. Evolutionary Biology, Vol 29 1996; 29:73-106.
16. Snook RR, Markow TA. Mating system evolution in sperm-heteromorphic Drosophila. J Insect Physiol 2001; 47:957-64; PMID:11472758; http://dx.doi.org/10.1016/S0022-1910(01)00070-1
17. Markow TA. Perspective: Female remating, operational sex ratio, and the arena of sexual selection in Drosophila species. Evolution 2002; 56:1725-34; PMID: 12389717
18. Rodríguez-Muñoz R. A. Bretman IA, Slate J, Walling CA, Tregenza T. Natural and sexual selection in a wild insect population. Science 2010; 328:1269-72; PMID:20522773; http://dx.doi.org/10.1126/science.1188102
19. House CM, Walling CA, Stamper CE, Moore AJ. Females benefit from multiple mating but not multiple mates in the burying beetle *Nicrophorus vespilloides*. J Evol Biol 2009; 22:1961-6; PMID:19682308; http://dx.doi.org/10.1111/j.1420-9101.2009.01800.x
20. Arnqvist G, Nilsson T. The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 2000; 60:145-64; PMID:10973716; http://dx.doi.org/10.1006/anbe.2000.1446
21. Gowaty PA. Beyond extra-pair paternity: individual constraints, fitness components, and social mating systems. In: Lucas J, Simmons L, eds. Essays on animal behavior: Celebrating 50 years of animal behaviour. Cambridge University: Cambridge, 2006: 221-56.
22. Firman RC, Cheam LY, Simmons LW. Sperm competition does not influence sperm hook morphology in selection lines of house mice. J Evol Biol 2011; 24:856-62; PMID:21306461; http://dx.doi.org/10.1111/j.1420-9101.2010.02219.x
23. Firman RC, Simmons LW. Sperm competition and the evolution of the sperm hook in house mice. J Evol Biol 2009; 22:2505-11; PMID:19878408; http://dx.doi.org/10.1111/j.1420-9101.2009.01867.x
24. Markow TA, O'Grady P. Drosophila: A guide to species identification and use. Academic Press, 2005.
25. Bundgaard J, Barker JSF. Remating, sperm transfer, and sperm displacement in the cactophilic species Drosophila buzzatii Patterson & Wheeler (Diptera: Drosophilidae). Biol J Linn Soc Lond 2000; 71:145-64.
26. Markow TA, O'Grady P. Reproductive ecology of Drosophila. Funct Ecol 2008; 22:747-59; http://dx.doi.org/10.1111/j.1365-2435.2008.01457.x
27. Lacey Knowles L, Hernandez BB, Markow TA. Nonantagonistic interactions between the sexes revealed by the ecological consequences of reproductive traits. J Evol Biol 2005; 18:156-61; PMID:15669972; http://dx.doi.org/10.1111/j.1420-9101.2004.00779.x
28. Markow TA. A comparative investigation of the mating system of *Drosophila hydei*. Anim Behav 1985; 33:775-81; http://dx.doi.org/10.1016/S0003-3472(85)80010-5
29. Bateman AJ. Intra-sexual selection in *Drosophila*.. Heredity 1948; 2:349-68; PMID:18103134; http://dx.doi.org/10.1038/hdy.1948.21
30. Tang-Martinez Z, Ryder TB. The problem with paradigms: Bateman's worldview as a case study. Integr Comp Biol 2005; 45:821-30; PMID:21676833; http://dx.doi.org/10.1093/icb/45.5.821
31. Dewsbury DA. The Darwin-Bateman paradigm in historical context. Integr Comp Biol 2005; 45:831-7; PMID:21676834; http://dx.doi.org/10.1093/icb/45.5.831
32. Snyder BF, Gowaty PA. A reappraisal of Bateman's classic study of intrasexual selection. Evolution 2007; 61:2457-68; PMID:17725639; http://dx.doi.org/10.1111/j.1558-5646.2007.00212.x
33. Platt JR. Strong inference. Science 1964; 146:347-53; PMID:17739513; http://dx.doi.org/10.1126/science.146.3642.347
34. Gowaty PA, Hubbell SP. Reproductive decisions under ecological constraints: It's about time. Proc Natl Acad Sci USA 2009; 106:10017-24; PMID:19528648; http://dx.doi.org/10.1073/pnas.0901130106
35. Dewsbury DA. Ejaculate cost and male choice. Am Nat 1982; 119:601-10; http://dx.doi.org/10.1086/283938
36. Parker GA, Baker RR, Smith VGF. The origin and evolution of gamete dimorphism and the male-female phenomenon. J Theor Biol 1972; 36:529-53; PMID: 5080448; http://dx.doi.org/10.1016/0022-5193(72)90007-0
37. Trivers RL. Parental investment and sexual selection. In: Campbell B, ed. Sexual selection and the descent of man. Chicago: Aldine, 1972:136-79.
38. Darwin C. The descent of man and selection in relation to sex. London: John Murray, 1871.
39. Gowaty PA, Steinichen R, Anderson WW. Mutual interest between the sexes and reproductive success in Drosophila pseudoobscura. Evolution 2002; 56:2537-40; PMID:12583593; http://dx.doi.org/10.1111/j.0014-3820.2002.tb00178.x
40. Gowaty PA, Steinichen R, Anderson WW. Indiscriminate females and choosy males: Within- and between-species variation in Drosophila. Evolution 2003; 57:2037-45; PMID:14575325
41. Arnold SJ. Bateman principles and the measurement of sexual selection in plants and animals. Am Nat 1994; 144:S126-49; http://dx.doi.org/10.1086/285656
42. Rice WR. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature 1996; 381:232-4; PMID:8622764; http://dx.doi.org/10.1038/381232a0
43. Chapman T. Seminal fluid-mediated fitness traits in Drosophila. Heredity 2001; 87:511-21; PMID: 11869341; http://dx.doi.org/10.1046/j.1365-2540.2001.00961.x
44. Chapman T. The soup in my fly: Evolution, form and function of seminal fluid proteins. PLoS Biol 2008; 6:e179-82; PMID:18666830; http://dx.doi.org/10.1371/journal.pbio.0060179
45. Chapman T, Bangham J, Vinti G, Seifried B, Lung O, Wolfner MF, et al. The sex peptide of Drosophila melanogaster: Female post-mating responses analyzed by using RNA interference. Proc Natl Acad Sci USA 2003; 100:9923-8; PMID:12893873; http://dx.doi.org/10.1073/pnas.1631635100
46. Chapman T, Davies SJ. Functions and analysis of the seminal fluid proteins of male Drosophila melanogaster fruit flies. Peptides 2004; 25:1477-90; PMID: 15374649; http://dx.doi.org/10.1016/j.peptides.2003.10.023
47. Chapman T, Herndon LA, Heifetz Y, Partridge L, Wolfner MF. The Acp26Aa seminal fluid protein is a modulator of early egg hatchability in Drosophila melanogaster. Proc Biol Sci 2001; 268:1647-54; PMID:11506676; http://dx.doi.org/10.1098/rspb.2001.1684
48. Chapman T, Neubaum DM, Wolfner MF, Partridge L. The role of male accessory gland protein Acp36DE in sperm competition in Drosophila melanogaster. Proc Biol Sci 2000; 267:1097-105; PMID:10885514; http://dx.doi.org/10.1098/rspb.2000.1114
49. Gowaty PA. Sexual dialectics, sexual selection, and variation in mating behavior. In: Gowaty PA, ed. Feminism and Evolutionary Biology. New York: Chapman Hall, 1997:351-84.
50. Holland B, Rice WR. Perspective: Chase-away sexual selection: antagonistic seduction versus resitance. Evolution 1998; 52:1-7; http://dx.doi.org/10.2307/2410914
51. Markow TA, Coppola A, Watts TD. How Drosophila males make eggs: it is elemental. Proc Biol Sci 2001; 268:1527-32; PMID:11454298; http://dx.doi.org/10.1098/rspb.2001.1673
52. Markow TA, Ankney PF. Drosophila males contribute to oogenesis in a multiple mating species. Science 1984; 224:302-3; PMID:17734916; http://dx.doi.org/10.1126/science.224.4646.302
53. Roughgarden J, Oishi M, Akcay E. Reproductive social behavior: cooperative games to replace sexual selection. Science 2006; 311:965-9; PMID:16484485; http://dx.doi.org/10.1126/science.1110105
54. Bono JM, Matzkin LM, Kelleher ES, Markow TA. Postmating transcriptional changes in reproductive tracts of con- and heterospecifically mated Drosophila mojavensis females. Proc Natl Acad Sci USA 2011; 108:7878-83; PMID:21518862; http://dx.doi.org/10.1073/pnas.1100388108
55. Moore AJ, Pizzari T. Quantitative genetic models of sexual conflict based on interacting phenotypes. Am Nat 2005; 165:S88-97; PMID:15795865; http://dx.doi.org/10.1086/429354
56. Hrdy SB. Infanticide among animals: A review, classification, and examination of the implications for the reproductive strategies of females. Ethol Sociobiol 1979; 1:13-40; http://dx.doi.org/10.1016/0162-3095(79)90004-9
57. Hrdy SB. The woman that never evolved. Cambridge: Harvard University Press, 1981.
58. Eberhard EB. Female control: Sexual selection by cryptic female choice. Princeton: Princeton University Press, 1996.
59. Gowaty PA. Architects of sperm competition. Trends Ecol Evol 1994; 9:160-2; PMID:21236807; http://dx.doi.org/10.1016/0169-5347(94)90076-0
60. Clark AG, Begun DJ. Female genotypes affect sperm displacement in Drosophila. Genetics 1998; 149:1487-93; PMID:9649536
61. Clark AG, Begun DJ, Prout T. Female x male interactions in Drosophila sperm competition. Science 1999; 283:217-20; PMID:9880253; http://dx.doi.org/10.1126/science.283.5399.217
62. Gowaty PA. Reproductive compensation. J Evol Biol 2008; 21:1189-200; PMID:18564347; http://dx.doi.org/10.1111/j.1420-9101.2008.01559.x
63. Gowaty PA, Anderson WW, Bluhm CK, Drickamer LC, Kim YK, Moore AJ. The hypothesis of reproductive compensation and its assumptions about mate preferences and offspring viability. Proc Natl Acad Sci USA 2007; 104:15023-7; PMID:17848509; http://dx.doi.org/10.1073/pnas.0706622104
64. Gowaty PA, Hubbell SP. Chance, time allocation, and the evolution of adaptively flexible sex role behavior. Integr Comp Biol 2005; 45:931-44; PMID:21676844; http://dx.doi.org/10.1093/icb/45.5.931
65. Brown WD, Bjork A, Schneider K, Pitnick S. No evidence that polyandry benefits females in *Drosophila melanogaster*.. Evolution 2004; 58:1242-50; PMID: 15266973
66. Hosken DJ, Stockley P. Benefits of polyandry: A life history perspective. Evol Biol 2003; 33:173-94.
67. Gowaty PA, Kim YK, Rawlings J, Anderson WW. Polyandry increases offspring viability and mother productivity but does not decrease mother survival in Drosophila pseudoobscura. Proc Natl Acad Sci USA 2010; 107:13771-6; PMID:20643932; http://dx.doi.org/10.1073/pnas.1006174107
68. Priest NK, Galloway LF, Roach DA. Mating frequency and inclusive fitness in Drosophila melanogaster. Am Nat 2008; 171:10-21; PMID:18171147; http://dx.doi.org/10.1086/523944

69. Gowaty PA, Hubbell SP. Killing time: A mechanism of sexual selection and sexual conflict. In: Leonard J, Cordoba-Aguilar A, eds. Primary Sexual Characters in Animals Oxford: Oxford University Press, 2010:79-96.
70. Anderson WW, Kim YK, Gowaty PA. Experimental constraints on mate preferences in Drosophila pseudoobscura decrease offspring viability and fitness of mated pairs. Proc Natl Acad Sci USA 2007; 104:4484-8; PMID:17360550; http://dx.doi.org/10.1073/pnas.0611152104
71. Hubbell SP, Johnson LK. Environmental variance in lifetime mating success, mate choice, and sexual selection. Am Nat 1987; 130:91-112; http://dx.doi.org/10.1086/284700
72. Kim y-K, Weber MB, Anderson WW, Gowaty PA. No evidence of intrinsic quality differences between preferred and non-preferred Drosophila pseudoobscura in tests of mate preferences. Journal of Integrative Zoology 2010. In press.
73. West-Eberhard MJ. Developmental plasticity and evolution. Oxford: Oxford University Press, 2003.
74. Markow TA. "Cost" of virginity in wild Drosophila melanogaster females. Ecology and Evolution 2011; http://dx.doi.org/10.1002/ece3.54